6052

# The effect of aeration, initial inoculum and meat microflora on the growth kinetics of *Listeria monocytogenes* in selective enrichment broths

*The effect of increased aeration, initial inoculum and the meat microflora on the growth kinetics of* Listeria monocytogenes *in meat samples enriched in* Listeria *selective broth were investigated. Growth rate and lag phase duration were calculated following linear regression analysis on the growth data. Neither aeration or variations in the initial inoculum levels altered the length of the lag phase or the growth rate of* L. monocytogenes. *The growth kinetics of the meat microflora was similar under all of the experimental conditions investigated. Overall the meat microflora was shown to have a faster growth rate than* L. monocytogenes *in selective media. Organisms growing during* Listeria *selective enrichment were identified as* Lactobacillus plantarum, Lactobacillus delbrueckii *and pseudomonads. The implications of the growth of these organisms in* Listeria *selective broths are discussed.*

## Introduction

The detection of pathogens from selective enrichment broths is an important step in conventional isolation methods. It is also important for many rapid methods which require sample enrichment to grow numbers of the target pathogen to detectable levels. Such methods include immunomagnetic techniques (Skjerve et al.1990, Blackburn et al. 1991), flow cell cytometry (Boye et al. 1983, Donnelly et al. 1986), electrical methods (Bolton 1990, Ogden 1990), enzyme linked immunoasorbent assays (Mattingly et al. 1988, Walker et al. 1990) and DNA hybridization techniques (Rossen et al. 1991, Wernars et al. 1991).

Little work has been carried out to determine the growth kinetics of *Listeria monocytogenes* in commonly used enrichment media. Different workers have recommended various enrichment broths as being the most suitable for the isolation of *L. monocytogenes* from food samples with large numbers of competing microflora (Doyle and Schoeni 1986, Lammerding and Doyle 1989, van Netten et al. 1989, Yu and Fung 1991). Few of these recommendations are based on the growth kinetics of the organism in these broths.

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### *Aerated, stirred cultures*

Pure culture and inoculated meat systems were established a described above. Incubation condition were modified by pumping air into the flasks (Air pump, Jetto, Hong Kong) at a rate of 30·0 $l^{-1}$ min and stirred continuously at a speed of 1000 rev $min^{-1}$ using an electric stirrer (IKA-Werk, Janke & Kunkel, Germany). Other incubation conditions were as previously described.

### *Effect of aeration and initial meat micro-flora on* L. monocytogenes *growth*

Minced beef samples with initial TVCs ranging between $log_{10}$6–7 (6·04–6·97), 7–8 (7·12–7·90) or 8–9 (8·09–8·99) cfu $g^{-1}$ were prepared as above and inoculated with *L. monocytogenes* to achieve an initial level of 1·0 cfu $ml^{-1}$. All cultures were set up in duplicate. One was aerated with stirring and the other used as a static control. Samples were taken hourly and assayed for *L. monocytogenes* and TVC.

### *Effect of inoculum level on* L. monocytogenes *growth*

Four samples of minced beef (25.0 g) were mixed with *L. monocytogenes* suspensions (10·0 ml) containing 1, 25, 250 and 2500 cfu respectively. This yielded meat samples containing *L. monocytogenes* at concentrations of 1 25 $g^{-1}$, 1 $g^{-1}$, 10 $g^{-1}$ and 100 $g^{-1}$. The inoculated meat samples were placed in 225 ml of enrichment broth to give cultures containing *L. monocytogenes* at final concentrations of 0·004, 0·11 and 10 cfu $ml^{-1}$.

### *Growth of meat microflora in* Listeria *selective broths*

It was decided to identify the bacteria which were responsible for the increases in the TVC's during incubation in the selective enrichment broths (UVM I, Palcam). Microorganisms frequently isolated using identification methods described by Cowan and Steel (1965) included *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, pseudomonads and *Aeromonas*.

Further information on these four contaminants was acquired by determining the growth characteristics for these four organisms in *Listeria* selective enrichment broths. 10 cfu of each of the organisms were added to 10·0 ml of 0·1% peptone water using the procedure previously described and the suspension was added to 225 ml *Listeria* selective enrichment broth. During incubation, 1·0 and 0·1ml aliquots were pipetted at regular intervals from the culture system and plated onto PCA (Oxoid), MRS agar (Oxoid), *Pseudomonas* agar (CFC, Oxoid) and starch ampicillin agar (Palumbo et al. 1985) to count total viable bacteria, the lactobacilli. *Pseudomonas* and *Aeromonas* respectively. The PCA plates, MRS plates and *Pseudomonas* agar plates were incubated at 25°C for 3 days. The starch ampicillin agar plates were incubated anaerobically at 30°C for 2 days. Colonies were counted and the number of *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Pseudomonas* spp. and *Aeromonas* in the culture were calculated.

*Lactobacillus delbrueckii* and *Lactobacillus plantarum* were distinguishable on the MRS plates due to the differences in the morphology of the colonies for these two organisms.

*Lactobacillus plantarum* being large (3–4 mm) cream coloured raised colonies and *Lactobacillus delbrueckii* a small pinpoint (0·5–1 mm) rough textured, white colony. When inoculated into pure cultures of *Listeria* selective broths no growth of either of the *Lactobacilli* occurred. It was deemed that the meat contained essential growth factors for lactobacilli (Sharpe and Fryer 1965). It was necessary to obtain meat which was free of lactobacilli, pseudomonads and *Aeromonas* to carry out the growth curve studies.

Samples of minced beef (25·0 g) were autoclaved at 121°C for 15 min. The samples were mixed with inocula (10 ml) containing *Lactobacillus plantarum* (25 cfu $ml^{-1}$), *Lactobacillus delbrueckii* (25 cfu $ml^{-1}$), *Pseudomonas* isolate (25 cfu $ml^{-1}$, and *Aeromonas* (25 cfu $ml^{-1}$) and placed in 225 ml *Listeria* selective enrichment broth (UVM I or Palcam) giving a final inoculum of 1·0 cfu $ml^{-1}$ of each species in the culture. The cultures were incubated at 30°C ±1°C for 32 h.

All the above experiments were carried with the addition of *Listeria monocytogenes* (1 cfu $ml^{-1}$) to ascertain if these organisms had any effect on the growth of the pathogen.

### *Statistical analysis*

In this study, the growth curves were generated using regression analysis. The growth kinetics calculated included specific growth rates and the lag phase durations.

These were calculated as described by Broughall et al. (1983). All data was included that occurred between the point that the initial inoculum level had increased by 50% until the point that the population density ceased to

When facultatively anaerobic organisms such as *Listeria monocytogenes* grow in enrichment cultures they very quickly use up the available oxygen in the environment and a shift to anaerobic respiration will then occur. It was considered that by maintaining aerobic respiration which gives a much higher energy yield than anaerobic respiration, that a faster growth rate could be obtained (Trivett and Meyer 1971, George and Lund 1992).

However, others such as Schiemann and Olsen (1984) have reported that aeration has no effect on growth.

When predicting the growth of a pathogen, such as *L. monocytogenes*, in an enriched meat system, a number of factors may influence growth. These include the effect of the initial *L. monocytogenes* inoculum and whether the organism can compete with a large microflora on the food sample (Lanciotti 1992).

In this study, an investigation was carried out to determine the growth kinetics (growth rates and lag phase durations) of *L. monocytogenes* in two commonly used enrichment broths. Experiments were carried out both in pure culture and in the presence of the normal microflora of the meat. The influence of the initial inoculum level of *L. monocytogenes*, and aeration on the growth of *L. monocytogenes* were also examined.

## Materials and Methods

### *Meat sample*

Beef mince samples were obtained from local butcher shops, supermarkets or from the abattoir of the Industrial Development Unit (IDU) on site. All samples used were examined for the presence of naturally occurring *Listeria* using the USDA-FSIS procedure (McClain and Lee 1988).

### L. monocytogenes *culture*

*L. monocytogenes* serotype 4b (NCTC 11994) was obtained from the National Collection of Type Cultures at the Central Public Health Laboratory, Colindale, London.

### L. monocytogenes *inoculum*

*L. monocytogenes* was streaked onto tryptone soya agar (TSA) (Oxoid, Hants, UK) and incubated overnight at 30°C. An isolated colony was picked from the TSA plate using a sterile loop and dispersed into 9·0 ml of 0·1% peptone water (Difco, Detroit, MI, USA). The number of *L. monocytogenes* cells per ml of suspension was determined using a membrane filtration epifluorescent technique (Walls et al. 1989). The suspension was diluted in peptone water (0·1%) to give the desired inoculum levels.

All experiments in these investigations were replicated on three occasions.

### *Pure and meat culture systems*

Pure culture system were prepared by adding 10·0ml of inoculum containing *L. monocytogenes* (23 cfu $ml^{-1}$ to 225 ml of *Listeria* selective broth (UVM I, Oxoid) or Palcam broth (Lab M, Bury, UK) in a sterile flask. To prepare a meat culture system, a sample of beef mince (25·0 g) was mixed with 10·0 ml of *L. monocytogenes* inoculum containing 25 cfu $ml^{-1}$. The inoculated meat sample was placed in a sterile flask with 225 ml of enrichment broth (UVM I or Palcam). The final inoculum in both cultures was approximately 1·0 cfu $ml^{-1}$. Both pure and meat culture systems were incubated in a thermostatically controlled water-bath (AGB Scientific, Dublin) at 30 ±1°C for 32 h.

### *Generation of growth curves*

At hourly intervals during incubation of the pure or meat culture 1·0 or 0·1 ml aliquots were pipetted from the culture and plated onto *Listeria* selective agar (Palcam agar, Lab M). The 1·0 ml plates were dried for 1h in a laminar flow cabinet (Nuaire class II A) prior to incubation at 30°C for 48 h. Typical *Listeria* colonies were counted and the number of *L. monocytogenes* per ml of culture were calculated. In the case of the meat cultures five representative colonies from each sampling time were confirmed as *L. monocytogenes* (McLauchlin 1987).

In addition, 1·0 and 0·1 ml aliquots were pipetted from the meat culture systems at hourly intervals and plated onto plate count agar (Oxoid). The plates were incubated at 25°C for 3 days and the total viable count (TVC) calculated.

increase. Specific growth rates were determined from the slopes of the regression line. The length of the lag phases were determined by substituting the initial inoculum values in to the regression equation and solving.

The regression equation

$$Y = MX + C \quad (1)$$

was rearranged to

$$X = \frac{Y - C}{M} \quad (2)$$

where:
$X$ = time (predicted lag phase value) (h);
$Y$ = population density (initial inoculum) [$\log_{10}$ cfu ml$^{-1}$];
$C$ = intercept;
$M$ = slope.

F-tests and $t$-tests were used to compare the specific growth rates and lag phase durations obtained for *L. monocytogenes* and TVC's under different cultural conditions.

The $t$-tests were as follows:

$$t = \frac{\text{mean}_1 - \text{mean}_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}. \quad (3)$$

Because the predicted lag phase durations were independent variables the standard errors were calculated from the following equation at 95% confidence limits

$$SE = \frac{\hat{X} \pm \frac{t(RSD)}{b}\sqrt{(1+\frac{1}{n})(1-C)^2 + \frac{(\hat{X}-\bar{X}^2)}{\Sigma(X-\bar{X})^2}}}{1-C^2} \quad (4)$$

where:
$t$ = T value in the tables with $n$-2 degrees of freedom;
$X$ = lag phase duration;
$RSD$ = residual standard deviation;
$b$ = regression coefficient;
$n$ = number of replicates;
$C$ = $\frac{t \text{ (standard error of } b)}{b}$.

This is not a symmetrical standard error but when the value for $b$ (regression coefficient) is close to 1, the value of $C$ becomes small and the above equation can be simplified to give a symmetrical standard error (Snedelor and Cochran 1967).

$$\pm \frac{(RSD)}{B}\left(\sqrt{1+\frac{1}{n}}\right). \quad (5)$$

## Results

Growth curves generated for *L. monocytogenes* in all pure and meat culture systems were similar. The line of best fit was linear and the correlation coefficients ($r^2$) varied between 0·94 and 0·98.

No significant differences were observed between specific growth rate or lag phase duration for non-aerated and aerated, stirred cultures in pure or meat systems (Table 1). It was also shown that the initial TVC ($\log_{10}$6–9) of the meat had no significant effect on the growth of *L. monocytogenes* (Table 2). There were no significant differences between the growth rates and lag phase durations for the two selective media.

The effect of different initial inoculum levels on the growth kinetics of *L. monocytogenes* is shown in Table 3. The growth rates for the different inocula were very uniform and differences between them were non-significant. More substantial differences were noted among the observed lag times, depending on the initial inoculum levels. Nevertheless, these differences were non-significant, indicating a high degree of variability

**Table 1. The effect of aeration on the growth kinetics of *Listeria monocytogenes* in meat and pure culture systems in UVM I broth.**

| Growth conditions | Pure culture | Meat culture | $t$-test |
|---|---|---|---|
| Specific growth rate ($\log_{10}$cfu ml$^{-1}$ h$^{-1}$) | | | |
| Non-aerated | 0·28 | 0·46 | NS |
| Aerated | 0·30 | 0·45 | NS |
| $t$-test | NS | NS | |
| Lag time (h) | | | |
| Non-aerated | 4·01 | 4·62 | NS |
| Aerated | 3·96 | 4·51 | NS |
| $t$-test | NS | NS | |

Standard error of differences between means for: (1) growth rate = ± 0·08; (2) lag time = ±0·54.
Degrees of freedom = 8.
NS, Non-significant.

**Table 4. The effect of different enrichment media and initial total counts on the growth kinetics of the meat microflora.**

| Enrichment media | Initial meat microflora ($log_{10}$cfu $g^{-1}$) 6–7 | 7–8 | 8–9 | F-test |
|---|---|---|---|---|
| Specific growth rate ($log_{10}$cfu $ml^{-1}$ $h^{-1}$) | | | | |
| UVM I | 0·84 | 0·86 | 0·54 | NS |
| PALCAM | 0·75 | 0·67 | 0·65 | NS |
| Lag time (h) | | | | |
| UVM I | 1·78 | 2·58 | 1·89 | NS |
| PALCAM | 1·27 | 1·07 | 1·89 | NS |

Standard error of differences between means: (1) growth rate = ± 0·13; (2) lag time = ±0·49.
Degrees of freedom: residual = 6; treatment = 8.
NS, Non-significant.

**Table 5. Comparison of the growth kinetics of the meat microflora and *Listeria monocytogenes* in meat cultures.**

| Enrichment broth | TVC | *L. monocytogenes* | *t*-test |
|---|---|---|---|
| Specific growth rate ($log_{10}$cfu $ml^{-1}$ $h^{-1}$) | | | |
| UVM I | 0·75 | 0·46 | $P<0·01$ |
| PALCAM | 0·70 | 0·42 | $P<0·01$ |
| *t*-test | NS | NS | |
| Lag phase (h) | | | |
| UVM I | 2·08 | 4·62 | $P<0·001$ |
| PALCAM | 1·41 | 3·99 | $P<0·001$ |
| *t*-test | NS | NS | |

Standard error of differences for: (1) growth rate = *Listeria* = ± 0·06; TVC = ±0·04.
(2) lag time *Listeria* = ±0·47; TVC = ±0·50.
Degrees of freedom = 8.
NS, Non-significant.

not grow though it did survive the enrichment procedure. It was also shown that in the presence of these contaminants the growth rate of *Listeria* was similar to that observed for the total flora growing in meat systems as shown in Table 5.

## Discussion

This study was carried out to determine the growth kinetics of *L. monocytogenes* in commonly used selective enrichment media. In particular, the use of enrich-

**Table 6. Individual and combined growth rates ($log_{10}$cfu $ml^{-1}$ $h^{-1}$) of *L. plantarum, L. delbrueckii, Pseudomonas* spp. and *Listeria monocytogenes* in two enrichment media after co-inoculation (1 cfu $g^{-1}$) into sterile cooked meat.**

| Bacterial species | Enrichment media UVM I | PALCAM |
|---|---|---|
| TVC | 0·77 | 0·68 |
| *L. plantarum* | 0·21 | 0·28 |
| *L. delbrueckii* | 0·28 | 0·25 |
| *Pseudomonas* | 0·17 | 0·12 |
| *Listeria monocytogenes* | 0·35 | 0·38 |

ment broths for the isolation of the pathogen from meat samples was investigated.

The study showed no difference between the growth curves obtained for *L. monocytogenes* in pure or meat culture systems. This would confirm the view that modelling systems to predict the growth of pathogens or spoilage organisms on foods can be obtained from experiments in pure cultures, using laboratory media (Gibson et al. 1988).

When aerobic or facultative anaerobic bacteria grow in any culture system, they metabolize the oxygen present in their immediate environment. Resultant reductions in oxygen levels can lead to a reduction in growth rate. The objective of aeration and stirring or agitation is to ensure that there is no such depletion of available oxygen and that high growth rates are maintained. However, in the present study aeration and stirring was without significant effect on the growth kinetics of *L. monocytogenes* in pure or meat culture systems, indicating that this is not a limiting factor in the enrichment cultures. Trivett and Meyer (1971) demonstrated that in a medium with iron present, the growth rate of *L. monocytogenes* was stimulated by shaking the culture. This has also been observed by George and Lund (1992) for this organism. In contrast, other studies have indicated that bacterial growth in culture is inhibited by aeration.

A series of studies carried out by Buchanan and Phillips (1990) and Buchanan and Klawitter (1990) have shown that lag times are reduced and generation times increased in anaerobic conditions, particularly under non-optimal growth conditions. The temperature and pH conditions were almost identical to those reported by George and Lund (1992). Studies on *Yersinia enterocolitica* have shown that aeration was without effect on growth (Schiemann and Olsen 1984).

The above discussion indicates some considerable confusion regarding the effect of aeration on growth. This may be related to the precise conditions for aeration. It is interesting to note that in the majority of the studies referred to above aeration was achieved by agitation but the amounts and extent of this are not stated. Where aerated cultures were used by Davies et al. (1991) compared to the present work, the volumes used were very different (30 l $min^{-1}$ vs 1·8 l $min^{-1}$). Clearly the effect of aeration/oxygenation requires further clarification.

In this study the lag phase appeared to be longer in systems with lower inoculum levels. This view would be in agreement with Wilson and Miles (1964) who reported longer lag phase durations when the inocula were small. However, statistical analysis of the results obtained in the current study showed no significant differences in the lag phase duration between cultures inoculated with different initial *Listeria* numbers. This result was obtained because of the large variability and high standard error associated with these experiments. Mackey and Kerridge (1990) also found that the size of the *Salmonella* inoculum had no significant effect on maximum growth rate or final cell concentration of the pathogen. Studies by Buchanan and Phillips (1990) and Lanciotti et al. (1992) also concluded that *L. monocytogenes* grew independently of the initial inoculum size.

In the present study the influence of the initial level of the total meat microflora and the growth of *L. monocytogenes* was investigated. The data showed that changes in the initial flora had no effect on the subsequent growth of *L. monocytogenes* in enrichment broths. It was also observed that the meat microflora had a significantly faster growth rate than *Listeria* but this did not

appear to interfere with the isolation of the pathogen.

Tran et al. (1990) also concluded that the level of aerobic microflora did not influence the subsequent isolation of *Listeria* in different enrichment broths. Dallas et al. (1991) stated that organisms growing in enrichment broths do not necessarily compete with *Listeria*.

Regarding the total bacterial flora growing during the selective enrichment of *Listeria monocytogenes*, it was determined that the organisms growing included *L. plantarum*, *L. delbrueckii* and pseudomonads. Other workers have judged the selectivity of detection procedures on the basis of contaminants occurring on the selective agar plates i.e. enterococci and staphylococci (Buchanan et al. 1987, van Cassiday et al. 1989, van Netten et al. 1989). The results of this present study demonstrate that a much larger and more varied number of contaminants are growing in the enrichment media.

For cultural detection methods the selectivity of detection is determined by the selectivity of the enrichment procedure and the plating method. However, for many rapid methods, selectivity is based on liquid enrichment and large numbers of contaminants may have significant implications.

Although in this study the large number of coselected organisms did not appear to have an effect on the growth of *L. monocytogenes*, other workers have reported that some of theses organisms can affect *Listeria* growth. The problems of the growth of enterococci and Gram-negative organisms including pseudomonads have been recognized for some considerable time and many proposed selective media for *Listeria* contain antibiotics to suppress these species. The effects of pseudomonads on the growth of *Listeria* are by no means clear. Farrag and Marth (1989) reported that at some temperatures, some *Pseudomonas* spp. stimulated the growth of *Listeria*. With other pseudomonads at different temperatures *Listeria* growth was suppressed. The reasons underlying these effects remain to be clarified. The presence and growth of lactobacilli as noted in this study may in some circumstances have important implications. Lactobacilli produce bacteriocins, some of which are active against *Listeria* spp. (Harris et al. 1989a). For example Harris et al. (1989b) reported large decreases (6–7 fold) in some *Listeria* populations in media supplemented with nisin. Such bacteriocins, secreted by contaminants of the type co-selected in selective broths in this study, would have the undesirable effect of suppressing the rate of growth and efficiency of recovery, of *Listeria* during the selective enrichment process. Modifications or supplementation of selective media may be necessary to address these issues.

## References


Blackburn, C., Patel, P. D. and Gibbs, P. A. (1991) Separation and detection of *Salmonella* using immunomagnetic particles. Leatherhead Food R.A., Research Report No. 684.

Bolton, F. J. (1990) An investigation of indirect conductimetry for detection of some food-borne bacteria. *J. Appl. Bact.* **69**, 655–661.

Boye, E., Steen, H. B. and Skarstad, K. (1983) Flow cytometry of bacteria: a promising tool in experimental and clinical microbiology. *J. Gen. Microbiol.* **129**, 973–980.

Broughall, J. M., Anslow, P. A. and Kilsby, D. C. (1983) Hazard analysis applied to microbial growth in foods. Development of mathematical models describing the effect of water activity. *J. Appl. Bact.* **55**, 101–110.

Buchanan, R. L., Stahl, H. G. and Archer, D. L. (1987) Improved plating media for simplified, quantitative detection of *Listeria monocytogenes* in foods. *Food Microbiol.* **4**, 269–275.

Buchanan, R. L. and Klawitter, L. A. (1990) Effects of temperature and oxygen on the growth of *Listeria monocytogenes* at pH 4·5. *J. Food Sci.* **55**, 1754–1756.
Buchanan, R. L. and Phillips, J. G. (1990) Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. *J. Food Protect.* **5**, 370–376.
Cassiday, P. K., Brackett, R. E. and Beuchat, L. R. (1989) Evaluation of ten selective direct plating media for enumeration of *Listeria monocytogenes* in hams and oysters *Food Microbiol.* **6**, 113–125.
Cowan, S. T and Steel, K. J. (1965) *Manual for the identification of medical bacteria*. Cambridge, Cambridge University Press.
Dallas, H. L., Tran, T. T., Poindexter, C. E. and Hitchens, A. D. (1991) Competition of food bacteria with *Listeria monocytogenes* during enrichment culture. *J. Food Safety* **11**, 293–301.
Davies, A., Blood, R. M. and Gibbs, P. A. (1991) Competitive growth in *Salmonella* pre-enrichment medium. Leatherhead Food R. A. Research Report No. 688.
Donnelly, C. W. and Baigent, G. J. (1986) Method for flow cytometric detection of *Listeria monocytogenes* in milk. *Appl. Environ. Microbiol.* **52**, 689–695.
Doyle, M. P. and Schoeni. J. L (1986) Selective enrichment procedure for isolation of *Listeria monocytogenes* from fecal and biological specimens. *Appl. Environ. Microbiol.* **51**, 1127–1129.
Farrag, S. A. and Marth, E. H. (1989) Behaviour of *Listeria monocytogenes* when incubated together with *Pseudomonas* species in tryptose broth at 7 and 13°C. *J. Food Protect.* **5**, 536–539.
George, S. M. and Lund, B. M. (1992) The effect of culture medium and aeration on growth of *Listeria monocytogenes* at pH 4·5. *Lett. Appl. Microbiol.* **15**, 49–52.
Gibson, A. M., Bratchell. N. and Roberts, T. A. (1988) Predicting microbial growth: growth responses of *Salmonella* in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.* **6**, 155–178.
Harris, L. J. Daeschel, M. A., Stiles, M. E. and Klaenhammer, T. R. (1989a) Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. *J. Food Protect.* **52**, 384–387.
Harris, L. J., Klaenhammer, T. R. and Fleming, H. P. (1989b) Sensitivity and resistance of *Listeria monocytogenes* to nisin. Annual Meeting of the Institute of Food Technology Chicago IL, June 25–29 Abstr. 354.
Lammerding, A. and Doyle, M. P. (1989) Evaluation of enrichment procedures for recovering *Listeria monocytoenes* from dairy products. *Int. J. Food Microbiol.* **9**, 249–268.
Lanciotti, R., Massa, S. Guerzoni, M. E. and Di Fabio, G. (1992) Light butter: natural microbial population and potential growth of *Listeria monocytogenes* and *Yersinia enterocolitica*. *Lett. Appl. Micicrobiol.* **15**, 256–258.
Mackey, B. M. and Kerridge, A.L. (1988) The effect of incubation temperature and inoculum size on growth of *Salmonella* in minced beef. *Int. J. Food Microbiol.* **6**, 57–65.
Mattingly, J. A., Butman, B. T., Planck, M. C., Durham, R. J. and Robison, B. J. (1988) Rapid monoclonal antibody based enzyme-linked immunosorbent assay for detection of *Listeria* in food products. *J. Assoc. Off. Anal. Chem.* **71**, 679–681.
McClain, D. and Lee, W. H. (1988) Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. *J. Assoc. Off. Anal. Chem.* **71**, 660–664.
McLauchlin, J. (1987) *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in humans. *J. Appl. Bact.* **63**, 1–11.
Murray, E. D. G., Webb, R. A. and Swann, M.B.R. (1926) A disease of rabbits characterised by a large mononuclear leucocytosis. caused by a hitherto undescribed bacillus, Bacterium monocytogenes. *J. Pathol. Bacteriol.* **29**, 407–439.
Ogden, I. D. (1990) Salmonella detection by a modified lysine conductance medium. *Lett. Appl. Microbiol.* **11**, 69–72.
Palumbo, S. A., Maxino, F., Williams, A. C., Buchanan, R. L. and Thayer, D. W. (1985) Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophilia*. *Appl. Environ. Microbiol.* **50**, 1027–1030.
Rossen, L., Holmstrom, O. J. E. and Rasmussen. O. F. (1991) A rapid polymerase chain reaction (PCR) based assay for the identification of *Listeria monocytogenes* in food samples. *Int. J. Food. Microbiol.* **14**, 145–152.
Schiemann, D. A. and Olsen, S. A. (1984) Antagonism by gram-negative bacteria to growth of *Yersinia enterocolitica* in mixed cultures. *Appl. Environ. Microbiol.* **48**, 539–544.

Sharpe, E. and Fryer, T. F. (1965) Media for lactic acid bacteria. *Laboratory Practice* **14**, 697–701.

Skjerve, E., Rorvik, L. M. and Olsvik, O. (1990) Detection of *Listeria monocytogenes* in foods by immunomagnetic separation. *Appl. Environ. Microbiol.* **56**, 3478–3481.

Snedelor, G. W. and Cochran, W. G. (1967) *Statistical methods*. Ames, Iowa State University Press.

Tran, T. T., Stephenson, P. and Hitchins, A. D. (1990) The effect of aerobic mesophilic microflora levels on the isolation of inoculated *Listeria monocytogenes* strain LM82 from selected foods. *J. Food Safety* **10**, 267–275.

Trivett, T. L. and Meyer E. A. (1971) Citrate cycle and related metabolism of *Listeria monocytogenes*. *J. Bacteriol.* **107**, 770–779.

van Netten, P., Pearles, I., van de Moosdijk, A., Curtis, G. D. W. and Mossel, D. A. A. (1989) Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* species. *Int. J. Food Microbiol.* **18**, 299–316.

Walker, S. J., Archer, P. and Appleyard, J. (1990) Comparison of the *Listeria*–Tek ELISA kit with cultural procedures for the detection of *Listeria* species in foods. *Food Microbiol.* **7**, 335–342.

Walls, I., Sheridan, J. J. and Levett, P. N. (1989) A rapid method of enumerating microorganisms from beef, using an Acridine orange Direct-Count Technique. *Ir. J. Food. Sci. Technol.* **3**, 23–31.

Wernars, K., Heuvelman, C. J., Chakraborty, T. and Notermans S. H. W. (1991). Use of the polymerase chain reaction for the direct detection of *Listeria monocytogenes* in soft cheese. *J. Appl. Bacteriol.* **70**, 121–126.

Wilson, G. and Miles, A. A. (1964) *Topley and Wilson's principles of bacteriology and immunity*, 5th edn., Vol.1. London, Edward Arnold.

Yu, L. S. L. and Fung, D. Y. C. (1991) Evaluation of FDA and USDA procedures for enumerating *Listeria monocytogenes* in ground beef. *Food Microbiol.* **8**, 69–74.